Supporting information for: Orthogonal modular gene repression in E. coli using engineered CRISPR/Cas9

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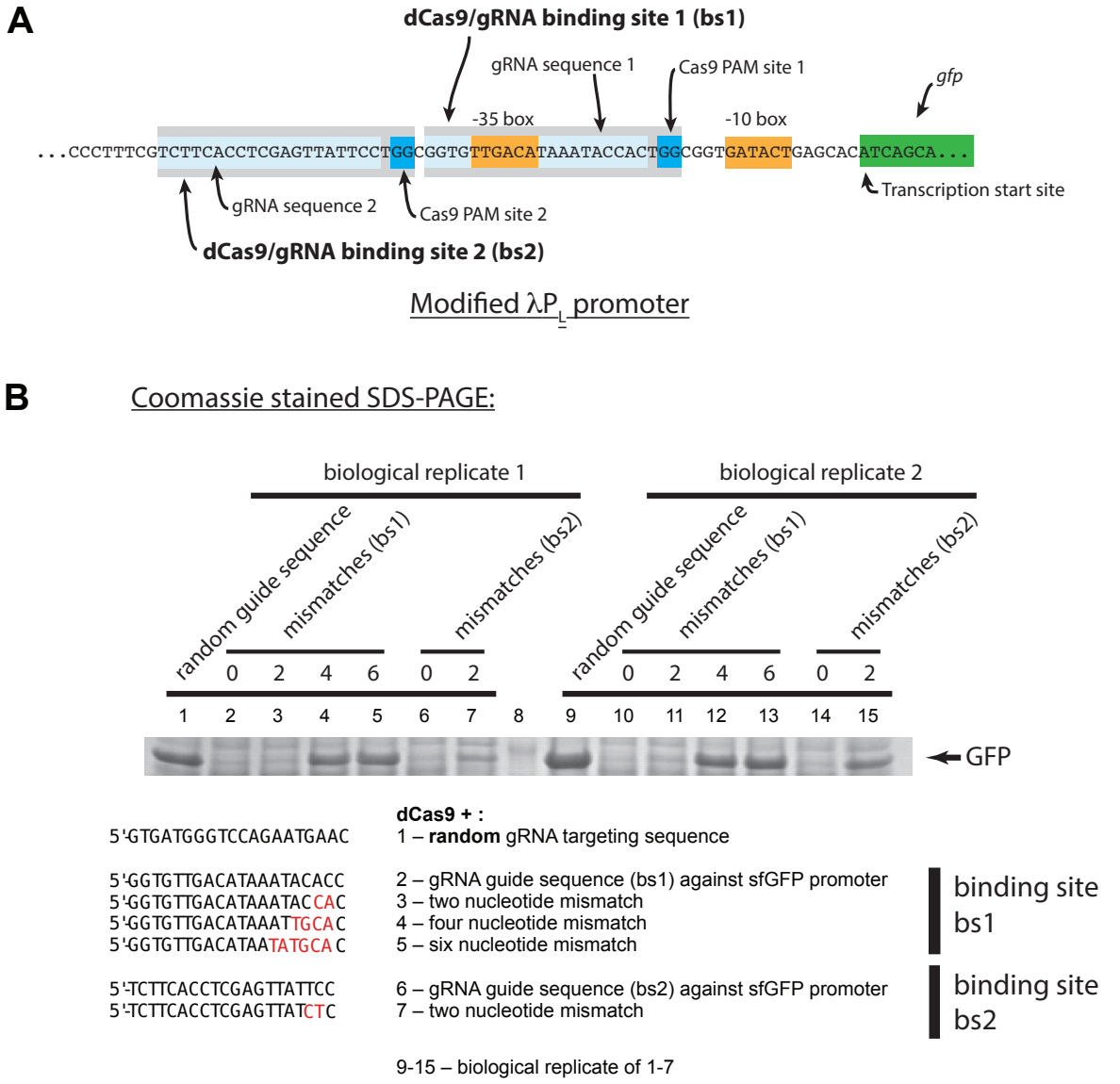

Figure S1: CRISPR/Cas9 repression of λP_L promoter. (A) Modified λP_L promoter used for gRNA sequence mismatch assay. Two dCas9/gRNA binding positions denoted as bs1 and bs2 were tested. (B) Mismatch assay results. Two mismatches between the target DNA and gRNA seed sequence were tolerated at promoter position bs1. Repression at position bs2 was less sensitive to mismatches within the corresponding gRNA seed sequence. In this experiment MBP-dCas9, TEV protease and gRNA were constitutively expressed from $P_{LtetO-1}$ promoter in *E. coli* JS006, otherwise the assay conditions were the same as described in Methods. GFP expression was analyzed using a standard Coomassie stained 10% SDS-PAGE gel.

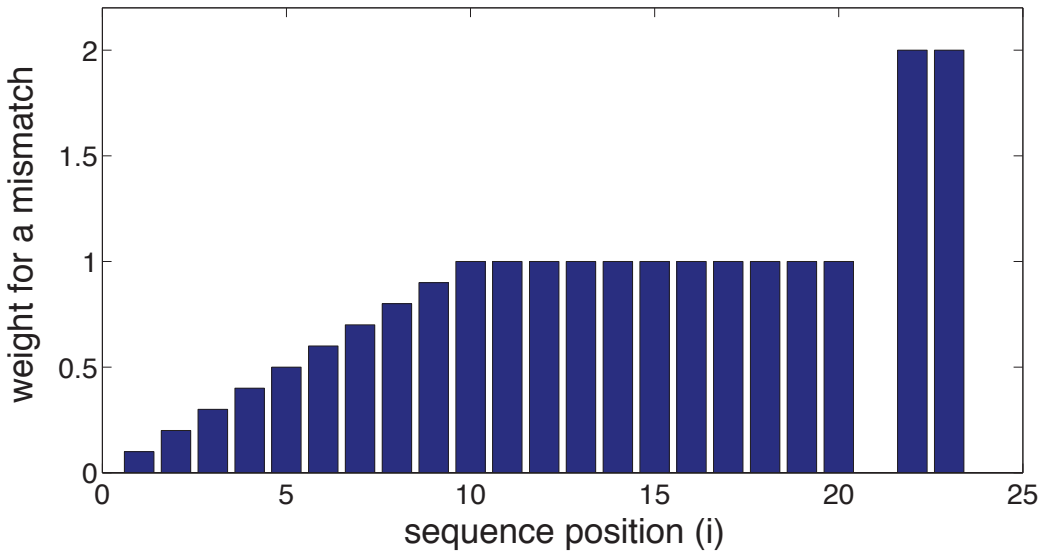

Figure S2: Weighting function used in the algorithm. The *S. pyogenes* protospacer adjacent motif (PAM) site NGG is at the positions 21-23.

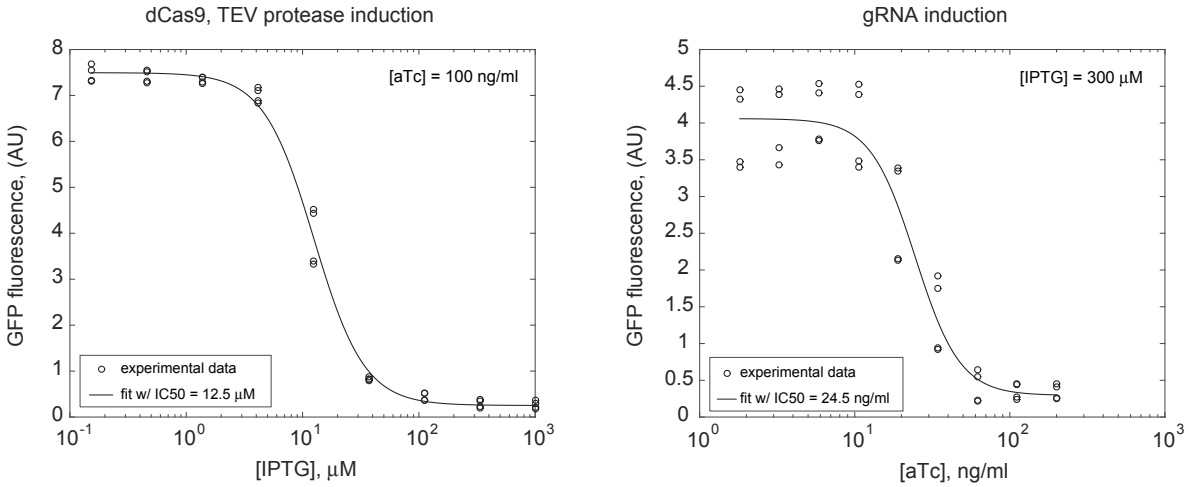

Figure S3: IPTG and aTc titration of a CRISPR/Cas9-regulatable promoter described in Fig. 1. The promoter and gRNA are based on the Sequence 1 (5'-TCTCAAGCTAGACTCTAGTG; see Fig. 1E). As in the experiments in Fig. 1, gRNA is produced from an aTc inducible promoter $P_{LtetO\text{-}1}$ while dCas9 and TEV protease are produced from an IPTG inducible promoter $P_{LlacO\text{-}1}$. The induction curves were fit with the standard Hill functions ($y = A/(1 + (x/IC_{50})^n) + B$), where n is the Hill coefficient and A, B, IC_{50} are determined from fitting. As a result the IPTG and aTc titration data were fit with $n = 2$, $IC_{50} = 12.5$ μM and $n = 3$, $IC_{50} = 24.5$ ng/ml respectively.

Table S1: The list of the top 20 most orthogonal gRNA guide sequences as found by the algorithm described in this paper.

Sequence number	**guide gRNA sequence (5'-3')**	**minimum weighted Hamming distance of orthogonal set**
1	TCTCAAGCTAGACTCTAGTG	n/a
2	ATCAGTGTGTACTAAGTACT	11.2
3	TGACTGAGCTAGTGTACTCT	10.0
4	GACACATCTTAGAGTATGTA	9.3
5	AAGTGAGTCTGAGCTTAGAT	9.1
6	TTTAGTAGTCTACTTAGATG	8.7
7	CTGAGTTGAGAGCTCACACT	8.6
8	ACTACTCTAGAATTGTAGCT	8.6
9	ACAAGATCTAGTGTGAGACT	8.3
10	AATCACTTAGTATGACTAGT	8.1
11	CTAGAATCTCACAGAGCTCT	8.0
12	AACTGAACACTAGTAGACTT	7.8
13	GTATACTAGACACTATGCTA	7.6
14	CTCAGCTAGTCTAGAGCACA	7.4
15	TGCATGCATTAGTACTAGCT	7.4
16	GCTTGACTTAGTCTAAACTA	7.2
17	GCTGCTAGAGTCTCTAAGTA	7.0
18	AGCTCAGTACTATACTAGAT	6.9
19	TGAGTAAGTAGACTCACATT	6.9
20	CAGTCATTACTACTCAGTCT	6.8